

REMARKS

This Amendment is filed in response to the Office Action dated September 20, 2002. By this Amendment, claims 2-3, 13-14, 17-22, 36-37, and 44-45 are canceled and claims 1, 4, 6, 12, 24, 39, 46, 48-49 and 57 are amended and new claim 64 is added; support for the amendments maybe found in the original claims as filed. Claims 1-60, and 64 are pending. New claim 64 is a rewritten form of originally filed claim 19 that further includes the limitations of originally filed claim 24. No new matter is added and the present claims have not been amended so as to encompass new subject matter or limitations that would require a new search.

The spelling of the terms "poly-L-lysine" and "neroaxis" have been changed (pages 15, and 1, respectively). The Examiner has asked for MAP2ab to be respelled as "MAP2" but a person of ordinary skill in the art will immediately recognize the term "MAP2ab", as indicated in published literature.

With regards to the Examiner's objections to the claims as discussed in section 7 of the Office Action, the Examiner is requested to withdraw the objection and examine the claims in light of the elected species, i.e., bFGF. In the event that a species claim is allowed, the genus claims should be examined in light of all of their species. MPEP 8th Ed., 809.02(a), ¶ 8.01 at page 800-49. The undersigned notes that claims 1, 6-10, 12, 32, 39-41, 43, 49-52, 54, and 57 are claims that are directed to the FGF family genus that has bFGF as a species and therefore these claims are to be examined with respect to all of the species of the FGF family if a claim to the elected bFGF species is allowed.

I. Indications and arguments of the Office Action

The Examiner has rejected claims 1-14, 17-22, 24, 32-33, and 36-59 under 35 U.S.C. 112 ¶ 1 for lack of enablement. The Examiner has indicated, in items 14, 15, 16, and 17 of the Office Action, that undue experimentation would be required to practice the full scope of the claimed invention. Office Action Sections, 14, 15, 16, and 17, are related to, respectively: astrocytes, glial cells, neurons, and multipotent cells.

In support of these positions, the Examiner has pointed to Example 6.1.1 as not providing experimental detail as to the isolation of stem cells so that one skilled in the art would be unable to practice the invention (Office Action, ¶ 10). The Examiner has also pointed out that Example 6.1.2 describes how to isolate neural stem cells from an animal but does not name what animal from which the cells are isolated (Office Action, ¶ 10). The Examiner has pointed out that the art teaches that adult mammalian brains contain stem cells but argues that the art does not teach how to isolate stem cells from all animals (Office Action, ¶ 11).

II. The invention is novel, unobvious, described by the Applicant, has utility, and would not have been expected to succeed by a person of ordinary skill in the art.

The Examiner has pointed out that the prior art does not teach or suggest an in vitro system for transdifferentiation of astrocytes into neurons, oligodendrocytes and multipotent cells (Office Action ¶ 13, also indicated in ¶ 12). In support of this argument, the Examiner has also indicated that the use of murine cells would not be predictive of success in humans (Office Action ¶ 13). Since the prior art does not teach or suggest the present claims, the present claims are novel and unobvious. The Examiner has reviewed the claims and recited utilities for the claims (Office Action ¶ 9). The Examiner has accepted the written description of the claims provided by the Applicant since the claims have been examined and no written description rejections have been made. Further, the Examiner has pointed out that one skilled in the art would not have a reasonable expectation of success when practicing the claimed invention

(Office Action ¶ 13, also indicated in ¶ 12). Since a person of ordinary skill in the art would not have a reasonable expectation of success when practicing the claimed invention, there is no motivation to combine prior art references to make the present invention.

III. A person of ordinary skill in the art is able to practice the invention without isolating stem cells.

The Examiner's references to Examples 6.1.1 and 6.1.2 and arguments that the isolation of stem cells is not within the ordinary skill of the art are not relevant since a person of ordinary skill in the art would immediately recognize how to practice the invention without the isolation of stem cells.

The independent claims 1, 12, 39, and 57, and their associated dependent claims require a culture of astrocytes or glial cells as a starting cell culture. Independent claim 49, which is drawn to a screening method, requires "cultured cells" as a starting point. Claims 50-56, which are all of the dependent claims for independent claim 49, require astrocytes as a starting point. Thus the presently claimed invention requires astrocytes or glial cells as a starting culture or "cultured cells" as a starting culture for a screening method. As pointed out in the specification, e.g., at page 12, section 5.1 "Establishment of an In Vitro Culture of Glial Cells", numerous protocols are known to persons of ordinary skill in the art for establishing glial cells, which include astrocytes. Moreover, commercial sources for cell lines are well known to those skilled in these arts (page 12, 2nd ¶).

IV. Claims 4-9, 38, 57, 59, and 64 are specifically directed to a specific species of growth factor, bFGF, and to specific species of cell, an astrocyte, so that a person of ordinary skill would not need to perform any experimentation to perform the claimed invention; therefore rejections based on an argument of undue experimentation are irrelevant to these claims.

A. Examiner's remarks and relevant law of undue experimentation.

The Examiner has indicated that undue experimentation would be required because of the large quantity of experimentation necessary to force astrocytes to differentiate into neuronal cells, the lack of direction/guidance presented in the specification regarding evaluating FGF-2 effects on astrocytes, the absence of working examples directed to the astrocytes that have become neuronal due to treatment with FGF-2, the complex nature of the invention, the unpredictability of the effects of a growth factor on all astrocytes, the breadth of the claims which fail to recite what type of neurons or multipotent cells would result from bFGF treatment (Office Action, item 14).

The Examiner has further noted that the term neurons covers a large variety of cells that are present in the central and peripheral nervous system, as well as various subtypes of neurons, and reasoned that undue experimentation would be required because the large quantity of experimentation necessary to identify distinguishing characteristics of each neuron subtype, the lack of direction/guidance presented in the specification regarding neuronal markers of specific subtypes (e.g., GAD, TH), the absence of working examples directed to neuronal characteristics (e.g., neurotransmitter production and release), the complex nature of the invention, the unpredictability of what type of neurons will be derived from differentiation of progenitors in culture, and the breadth of the claims which are not limited to "which neuronal cells are considered neurons" (Office Action ¶ 16).

Several factors, referred to as *Wands* factors, are legally required to be analyzed to determine if undue experimentation is required, including: (A) The breadth of the claims, (B) The nature of the invention, (C) The state of the prior art, (D) The level of one of ordinary skill, (E) the level of predictability in the art, (F) The amount of direction provided by the inventor;

(G) the existence of working examples, and (H) the quantity of experimentation needed to make and use the invention based on the content of the disclosure. MPEP 2164.01(a). These factors are analyzed in order, below.

B. Analysis of the Wands factors for claims 4-9, 38, 57, 59, and 64.

Claims 4-9, 38, 57, 59, and 64 are directed to culturing cells to produce neurons or oligodendrocytes, or multipotent cells from astrocytes using bFGF. The *Wands* factors are analyzed as follows:

(A) The breadth of the claims. The Examiner has argued that the breadth of the claims disfavor the Applicant because the claims fail to recite what type of neurons would result from bFGF treatment. It is unnecessary, however, to specify neuronal subtypes since a person of ordinary skill in the art would be able to practice the claimed invention and to produce cells recognizable as neurons merely by following the Applicant's instructions. The Applicant has not claimed to make a particular subtype of neurons, but to have made cells that are recognizable as neurons. Although others may invent new ways to use bFGF to make particular neuronal subtypes in the future, the Examiner must not require the present Applicant to immediately invent all the possible new inventions that might fall within the scope of the present claims. Similarly, persons of ordinary skill will recognize the type of cell referred to herein as a multipotent cell. Indeed, Moreshead et al. (Appendix A) and Imura et al. (Appendix B) use the term "stem cell" to indicate cells having multipotence despite lacking a complete knowledge of the stem cell types or subtypes, showing that persons of ordinary skill are able to recognize cells denoted by the term multipotent. This issue favors the Applicant.

- (B) The nature of the invention.** The Examiner has argued that the nature of the invention is complex. The claims, however, are limited to in vitro cultures and use techniques well known to those of ordinary skill in the art and a person of ordinary skill in the art would readily appreciate how to practice the invention after reading the Application, since there is no need to make unusual equipment or unusual protocols. Therefore the nature of the invention is not complex and this issue weighs in favor of the Applicant.
- (C) The state of the prior art.** The Examiner has not argued that the state of the prior art is a factor that disfavors the Applicant. The Examiner has found that there is no prior art for practicing the claimed invention. But the prior art teaches how to perform the various technical tasks that are required to practice the invention, a factor that weighs in favor of the Applicant.
- (D) The level of one of ordinary skill.** The Examiner has not argued that the state of the prior art is a factor that disfavors the Applicant, although the Examiner has noted that the prior art does not describe the invention. The inventor has a Ph.D. in the life sciences, a typical level of expertise for those performing or directing research in this field. The level of ordinary skill in the field is high, a factor that weighs in favor of the Applicant.
- (E) The level of predictability in the art.** The Examiner has argued that the level of unpredictability is high: "the unpredictability of the effects of a growth factor on all astrocytes" (Office Action, section 14). It is difficult to predict what effects a growth factor will have on a cell in the absence of any data about how that growth factor affects that cell, e.g., Rao 1999. But in the present case, there is not an absence of data: bFGF has been shown to be effective for astrocytes. bFGF has been shown to be effective for astrocytes and may reasonably be expected that bFGF will be

effective for astrocytes in the future. There are subtypes of astrocytes, and astrocytes share common features and biochemical characteristics so that the performance of one astrocyte is predictive of the performance of astrocytes in general.

Indeed, Morshead et al. have recently reported that GFAP-positive cells such as astrocytes exposed to bFGF (i.e., FGF-2) may become neural stem cells. See Appendix A. Therefore others have been able to use a related process to make multipotent cells that have the potential to become neurons or oligodendrocytes. This issue favors the Applicant.

- (F) The amount of direction provided by the inventor.** The Examiner has argued that there is a lack of direction/guidance presented in the specification regarding evaluating bFGF effects on astrocytes. In fact, the specification discusses in detail that the effect of bFGF on astrocytes, when bFGF is used as directed in the specification, is to produce neurons. This issue favors the Applicant.
- (G) The existence of working examples.** The Examiner has argued that there is an absence of working examples directed to the astrocytes that have become neuronal due to treatment with FGF-2. In fact, the specification sets forth detailed procedures, e.g., in sections 5.1-5.5 that outline the various steps and conditions for performing the claimed process. And Working Example 6.0, 6.1, 6.1.1, 6.1.2, 6.1.3 sets forth detailed conditions and procedures for performing the claimed process. The specification further provides results showing the successful outcome of the Working Example, wherein bFGF and astrocytes are specifically taught and used, as demonstrated in Figures 1, 2, and 3. This issue favors the Applicant.
- (H) The quantity of experimentation needed to make and use the invention.** The Examiner has indicated that a "large quantity of experimentation necessary to force astrocytes to differentiate into neuronal cells" is needed. A person of ordinary skill

in the art immediately recognizes that the term "astrocyte" refers to a limited set of specialized cells. And the specification provides methods, techniques, and results for making astrocytes into neuronal cells. Since the use of bFGF and astrocytes is claimed and taught in the specification, there is essentially no need for experimentation. A large amount of experimentation is not needed; instead, what is needed is for a person of ordinary skill to follow the methods provided in the Application.

Significantly the Examiner has not suggested that there are many astrocytic subtypes. Only a few subtypes would therefore need be tested, assuming that testing of subtypes were necessary. Indeed, even a "considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed" MPEP 8th Ed., 2164.06 at page 2100-181, quoting *In re Wands*. The guidance in the specification is extremely detailed, even to the point of describing appropriate controls, e.g., section 5.7. Therefore testing of astrocytic cell subtypes is within the acceptable amount of experimentation established in *Wands*.

Analysis of the *Wands* factors for claims 4-9, 38, 57, 59, and 64 shows that conclusory reasoning that is not directed specifically to the claims is not sufficient to support an argument of lack of enablement. Instead, careful analysis of the *Wands* factors shows that the Applicant is favored in every category. The Examiner is therefore requested to withdraw rejections of these claims based on a lack of enablement under 35 U.S.C. 112 ¶ 1.

V. Claims 46-48 are specifically directed to a specific species of growth factor, bFGF, and specific class of cell (glial) so that a person of ordinary skill would not need to perform significant amounts of experimentation to perform the claimed invention.

A. Examiner's remarks and relevant law.

The Examiner has reasoned that undue experimentation would be required because of the large quantity of experimentation necessary to test all the subtypes of glial cells, the lack of direction/guidance presented in the specification regarding evaluating bFGF effects on all subtypes of glial cells, the absence of working examples directed to glial cells (non-fetal) that have become neuronal via treatment with bFGF, the complex nature of the invention, the unpredictability of the effects of a growth factor on glial cells and the breadth to the claims which do not limit the type of neurons or multipotent cells that would result from bFGF treatment (Office Action, Item 15). The Examiner pointed to about 8 types of glial cells and indicated that glial cell lines were available (Office Action, item 15). The need to test every single subtype would contribute to a need to perform undue experimentation to practice the invention because many subtypes would require testing (Office Action, item 15).

An analysis of the Wands factors is the legal test for lack of enablement due to an alleged need for undue experimentation.

B. Analysis of Wands factors for claims 46-48.

Claims 46-48 are directed to exposing a culture of glial cells to bFGF to obtain neurons. The *Wands* factors are analyzed as follows:

(A) The breadth of the claims. The Examiner has argued that the breadth of the claims disfavor the Applicant because the claims fail to recite what type of neurons would result from bFGF treatment. As discussed in the previous *Wands* analysis, however, the number of neuronal subtypes is not relevant so that this issue favors the Applicant.

- (B) **The nature of the invention.** The Examiner that the nature of the invention is complex. As discussed above, however, the invention is not complex so that this issue weighs in favor of the Applicant.
- (C) **The state of the prior art.** As discussed in the previous Wands analysis, this issue weighs in favor of the Applicant.
- (D) **The level of one of ordinary skill.** As discussed in the previous Wands analysis, this factor weighs in favor of the Applicant.
- (E) **The level of predictability in the art.** The Examiner has argued that the level of unpredictability is high: "the unpredictability of the effects of a growth factor on all astrocytes" (Office Action, section 14) and "the unpredictability of the effects of a growth factor on glial cells" (Office Action, section 15). It may be difficult to predict what effects a growth factor will have on a cell in the absence of any data about how that type of growth factor affects that type of cell. But in the present case, there is not an absence of data: bFGF has been shown to be effective for astrocytes and may reasonably be predicted to be effective for other astrocytes and related cells, i.e., glial cells.

With the exception of microglia, all glial cells *share a common embryonic origin with neurons*. (Albert et al., page 1064, Appendix C). Since the astrocytes of the invention are believed to dedifferentiate into a multipotent cell before being redifferentiated into a neuron or other cell, it can be reasonably predicted that other cells that are in a similar class with astrocytes and share a common embryonic origin with astrocytes and with neurons will also be dedifferentiated and redifferentiated in a similar manner (Application, Summary, 2nd ¶). These data that serve to predict that glial cells can be transdifferentiated using bFGF.

In fact, Imura et al., see Appendix B, have recently found that some but not all GFAP expressing glial cells may be cultured to be stem cells that produce neurons, oligodendrocytes, and astrocytes. These data that serve to predict that glial cells can be transdifferentiated using bFGF. This issue favors the Applicant. Applicant notes that Imura et al. use the terms astrocytes, neurons, glia, and oligodendrocytes in a manner that shows that these are terms known to those of skill in these arts.

(F) The amount of direction provided by the inventor. The Examiner has argued that there is a lack of direction/guidance presented in the specification regarding evaluating bFGF effects on "all types" of glial cells. The specification discusses in detail the effect of bFGF on astrocytes, which are a type of glial cells. The specification discusses at length and in detail the appropriate steps for evaluating bFGF effects on all types of cells, e.g., in section 5.7. Indeed, the specification provides an entire process for screening, testing, and evaluating bFGF effects on cells. The Application thus gives an abundance of clear direction in this area. This issue favors the Applicant.

(G) The existence of working examples. The Examiner has argued that there is an absence of working examples directed to glial cells that have become neuronal due to treatment with bFGF. In fact, the specification sets forth detailed procedures, e.g., in sections 5.1-5.5 that outline the various steps and conditions for performing the claimed process. And Working Example 6.0, 6.1, 6.1.1, 6.1.2, 6.1.3 sets forth detailed conditions and procedures for performing the claimed process using a type of glial cell, an astrocyte.

The specification further provides results showing the successful outcome of the Working Examples, wherein bFGF and a type of glial cell, an astrocyte, is specifically taught and used, as demonstrated in Figures 1, 2, and 3.

"To make a valid rejection, one must evaluate all of the facts and evidence and state why one would not expect to be able to extrapolate that one example across the entire scope of the claims" MPEP 8th ed. 2164.02 at page 2100-177. In the present case, the working example is similar to the claimed scope because the working example is directed to an astrocyte, which is a type of glial cell. And since, as discussed already, the astrocytes and glial cells share a common origin with neuronal cells, there is a relationship between the success of an astrocyte and other types of glial cells so that the astrocyte cell example may be extrapolated across the entire scope of the claims.

For all of these reasons, this issue favors the Applicant.

(H) The quantity of experimentation needed to make and use the invention. The Examiner has indicated a need to test every glial cell subtype. In the first place, it is likely that the effect of a growth factor on one subtype is predictable for an effect on other subtypes and therefore the actual amount of experimentation will be minimized. The impact of experimental data on predictability has been discussed in the predictability section herein.

In the second place, there are a limited number of subtypes so that testing them all would not be an undue quantity of experimentation. Glial cells are a class of cells having five subclasses: oligodendrocytes, astrocytes, Schwann cells, ependymal cells, and microglia. (Albert et al., page 1064-5, Appendix C). The Examiner has pointed to further subtypes: fibrous, protoplasmic, radial, and SVZ. Thus a total of about nine types and subtypes have been indicated. One of the types, astrocytes, has already been tested. Therefore, if there were no predictability, then 8 cell types would be required for experimentation.

A person of ordinary skill in the art could rapidly test 8 cell types after reading the Application. Testing 8 cell types as directed in the Application would be a small task for a person with access to cell culture facilities. Indeed, a "considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed" MPEP 8th Ed., 2164.06 at page 2100-181, quoting *In re Wands*. Indeed, the guidance in the specification is extremely detailed, even to the point of describing appropriate controls, e.g., section 5.7. The testing of a mere few cell lines is within the acceptable amount of experimentation established in *Wands*.

This issue favors the Applicant.

Analysis of the *Wands* factors for claims 46-48 shows that a careful analysis of the *Wands* factors indicates that the Applicant is favored in every category. The Examiner is therefore requested to withdraw rejections of these claims based on 35 U.S.C. 112 ¶ 1.

VI. Claims 1, 10-12, 32, 40-43 are directed to the FGF family and to specific species of cell, an astrocyte, so that a person of ordinary skill would not need to perform undue experimentation to perform the claimed invention.

Claims 1, 10-12, 32, and 40-43 are directed to culturing a specific species of cell, an astrocyte, with a member of the FGF family to thereby make neurons or oligodendrocytes. The claims recite the FGF family, which is generic to the presently selected species of the FGF family, which is bFGF. The Examiner's remarks are set forth in section IV above, which discusses claims 4-9, 38, 57, and 59. In brief, the claims were rejected under 35 U.S.C. 112 ¶ 1 for a lack of enablement due to an alleged need for undue experimentation. The Examiner's arguments related to claims 1, 10-12, 32, and 40-43 are the same as directed to claims 4-9, 38, 57,

and 59 so that the same analysis is applicable as set forth in Section IV. In brief, a point-by-point analysis of the Wands factors shows that the factors favor the Applicant.

VII. Claims 39 is directed to the FGF family and to specific class of cell, a glial cell, so that a person of ordinary skill would not need to perform undue experimentation to perform the claimed invention.

Claim 39 is directed to culturing a specific class of cell, a glial cell, with a member of the FGF family to thereby make neurons or oligodendrocytes. The claims recite the FGF family, which is generic to the presently selected species of the FGF family, which is bFGF. The Examiner's remarks are set forth in section V above, which discusses claims 46-48, and 59. In brief, the claims were rejected under 35 U.S.C. 112 ¶ 1 for a lack of enablement due to an alleged need for undue experimentation. The Examiner's arguments related to claims 46-48, and 59 are the same as directed to claim 39 so that the same analysis as set forth in section V is applicable. In brief, a point-by-point analysis of the *Wands* factors shows that the factors favor the Applicant.

VIII. Claims 49-56 are directed to a method of screening cells using the FGF family and glial cells, so that a person of ordinary skill would not need to perform undue experimentation to perform the claimed invention.

Claims 49-56 claim a method of screening cells using the FGF family and glial cells. As discussed below, a person of ordinary skill in the art will immediately understand how to make and use the claimed method after reading the Application.

A person of ordinary skill in the art would immediately understand from the specification and claims how to practice the claimed screening method, which involves culturing cells and exposing them a factor, with a culture of cells exposed to a member of the FGF family serving as a control (step h).

Specifically, a person of ordinary skill in the art would be able to grow cultured cells in vitro that included a first cell type but not a second cell type (step a), dissociate the cultured cells (Step b), replate the dissociated cells into a test well (step c), add a test factor a test well (step d), grow the cells in the test well in the presence of the test factor (step e), subsequently grow the cells in the absence of the test factor (step f), examine the cells for a second cell type (step g) and, run a control experiment using a member of the FGF family (step h). These steps are described in the specification and in cell culture manuals, as discussed in the specification, e.g., at section 5.7. None of these steps would require experimentation to ascertain how to perform them.

Further, claim 53 is specifically limited to the culture of astrocytes with bFGF, with the second cell type being a neuron: this claim is directly exemplified by the sections 6.1.1 to 6.1.4. Therefore essentially no experimentation is required to practice this claim.

Since a person of ordinary skill in the art would immediately be able to practice the claimed invention without experimentation, the claims are enabled and the rejection for lack of enablement because of a need for undue experimentation is not appropriate.

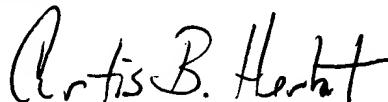
For all of these reasons, the Examiner is requested to withdraw these rejections of the claims.

IX. Conclusion

In view of the foregoing, it is submitted that this application is in condition for allowance. Favorable consideration and prompt allowance of the application are respectfully requested.

The Examiner is invited to telephone the undersigned if the Examiner believes it would be useful to advance prosecution.

Respectfully submitted,



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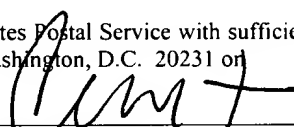
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Curtis B. Herbert, Ph.D., Esq.

ATTACHMENT
REDLINED AMENDMENT

In the Specification

Please substitute the following amended paragraph(s) and/or section(s):

Page 1, line 19

It has been reported that the entire ventricular [neuraxis] neroaxis, including the spinal cords of adult mammals, contain stem cells (Morshead and Van der Kooy, Journal of Neuroscience, 12:249-256, 1992; Reynolds and Weiss, Science, 255:1707-1710, 1992; Lois and Alvarez-Buyalla, Science, 264:1145-1148, 1994; Morshead et al., Neuron, 13:1071-1082, 1994; Weiss et al., Trends in Neuroscience, 19:387-393, 1996a, Journal of Neuroscience, 16:7599-7609, 1996 b). These stem cells may proliferate and expand in some circumstances and are affected by growth factors such as epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), leukemia inhibitor factor (LIF), and others; the stem cells may then differentiate into other cell types, including neurons, astrocytes, and oligodendrocytes (Reynolds and Weiss, 1992; Morshead et al., 1994; Weiss et al., 1996b). Recent data demonstrate that adult subventricular zone astrocytes or astrocyte progenitors can develop into stem cells in vivo (Doetsch, 1999).

Page 15, line 7

5.5 In vitro differentiation step of culturing cells

After the treatment step, the cells undergo an in vitro differentiation step that deprives the cells of the added factor used in the treatment steps. The in vitro differentiation step helps the cells transdifferentiate into various cell types including astrocytes, oligodendrocytes, and neurons. The cells may be cultured in various differentiation media that provide factors for cell survival. Suitable media includes a chemically defined medium such as DMEM/F12

supplemented with 1 μ M RA, 1 mM dbcAMP and 30 ng per ml BDNF (Medium I); 30 ng per ml BDNF (Medium II); or 20 ng per ml GDNF, 10 ng per ml FGF-8, and 100 μ M AA (Medium III). A medium supplemented with NEUROBASAL medium is also suitable (GIBCO). The cells are cultured on a substrate coated with laminin, [poly L-lysine] poly-L-lysine, polyornithine, a suitable extracellular matrix factor, or the like.

Claims As Amended

Please cancel claims 2-3, 13-14, 17-22, 36-37, and 44-45 without prejudice or disclaimer.

Please substitute the following amended claims for those currently pending:

1. (Once Amended) An in vitro method for producing neurons from astrocytes, the method comprising a culturing step of establishing a group of cells by culturing the astrocytes in vitro, and a subsequent treatment step of exposing the group of cells to at least one added factor that is a FGF family member such that neurons are produced as a result of the added factor.
4. (Once Amended) The method of claim [3] 1 wherein the FGF family member is bFGF.
6. (Once Amended) The method of claim 3 further comprising a subsequent in vitro differentiation step performed after exposing the group of cells to the at least one added factor, the in vitro differentiation step [being a step of] comprising culturing the group of cells without the added factor whereby the neurons are produced after the in vitro differentiation step.
12. (Once Amended) A method of producing a second cell type from astrocytes, the method comprising an initial culturing step of culturing the astrocytes and a subsequent treatment step of

contacting the astrocytes with an added factor, the added factor being at least one growth [factor(s)] factor chosen from the FGF family, and wherein the second cell type is a neuron or oligodendrocyte.

24. (Once Amended) The method of claim [22] 12 wherein the at least one added growth factor [includes] is FGF-2.

39. (Once Amended) A method of manipulating an in vitro culture of glial cells to produce a second cell type, the method comprising:

a culturing step of culturing a group of glial cells;

a dissociation step of dissociating the group of cells [prior to the treatment step]; and

a subsequent treatment step of contacting the group of cells with an added factor, the added factor including at least one growth factor chosen from the FGF family.

46. (Once Amended) The method of claim [45] 43 wherein the member of the FGF family is bFGF.

48. (Once Amended) The method of claim 47 further comprising an in vitro differentiation step, the in vitro differentiation step being [a step of] performed after contacting the group of cells with the added factor and comprising culturing the group of cells without the added factor.

49. (Once Amended) A method of screening growth factors for transdifferentiation, the method comprising the steps of:

- (a) growing cultured cells in vitro, including a first cell type but not a second cell type;
- (b) dissociating the cultured cells;
- (c) replating the cells into a plurality of test well means;
- (d) adding a test [growth] factor to the test well means;
- (e) growing the cells in the test well means in the presence of the test [growth] factor;
- (f) subsequently growing the cells in the test well means in the absence of the test
[growth] factor;
- (g) examining the cells to determine if cells of the second type are present; and

(h) Running a control experiment in other test well means using a member of the fibroblast growth factor family, wherein the first cell type is a glial cell and the second cell type is a neuron or oligodendrocytes.

57. (Once Amended) An in vitro method for producing neurons from astrocytes, the method comprising a culturing means for culturing astrocytes in vitro, and a subsequent treatment step of exposing the group of cells to at least one growth factor means, the growth factor means causing the production of neurons from the astrocytes and being chosen from the FGF family.

Please add new claim 64 as follows:

64. A method of producing a multipotent cell type from an astrocyte, the method comprising an initial culturing step of culturing the astrocytes and a subsequent treatment step of contacting the astrocytes with FGF.



APPENDIX A

SELECTIVE ABLATION OF GFAP POSITIVE CELLS IN THE ADULT SUBEPENDYMA RESULTS IN THE LOSS OF NEUROSPHERE FORMATION

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The cellular composition and organization of the adult germinal zone has been examined in detail, however the identity of the neural stem cell (nsc) has not been unequivocally established. To test if nsc's are a subpopulation of GFAP+'ve cells within the subependyma, we used transgenic (tg) mice expressing HSV-thymidine kinase from the GFAP promotor. Proliferating GFAP+'ve cells in the tg are ablated following exposure to ganciclovir (GCV). The germinal zones of adult tg and non-tg controls were dissected, dissociated and plated in the presence of EGF and FGF2. Tg animals gave rise to control numbers of spheres in the absence of GCV in vitro yet showed a 98% loss relative to controls in the presence of GCV in vitro. When GCV was infused unilaterally into the lateral ventricle for 3 days, non-tg mice generated control numbers of spheres (relative to saline infusion). However, a 98% loss in spheres was seen in tg animals infused with GCV. Since in vivo only exposure to GCV resulted in the same 98% loss seen when GCV was present in vitro, this suggests that a loss of GFAP+'ve progenitors (making up 99% of sphere cells) cannot account for the lack of sphere formation. To test whether proliferating GFAP+'ve cells are not nsc's but are necessary for their proliferation/survival, we plated tg cells in the presence of control GFP+'ve germinal zone cells at 1:1 and 1:10 ratios (tg:GFP). Even in the overwhelming presence of control cells tg cells did not form spheres following GCV exposure in vivo or in vitro. Our results suggest that nsc's are GFAP+'ve in the adult brain. Supported by: CIHR, CNIB



APPENDIX B

THE PREDOMINANT NEURAL STEM CELL ISOLATED FROM POSTNATAL PERI - VENTRICULAR GERMINAL ZONE IS A GFAP - EXPRESSING GLIA
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Peri-ventricular germinal zone (GZ) of adult brain contains multipotent neural stem cells (NSCs) whose cellular identity, derivation and regulation are uncertain. We used tissue culture techniques and transgenic mice expressing HSV-TK from the GFAP promoter to (i) test the hypothesis that GFAP-expressing glia are multipotent neural stem cells that generate neurons, oligodendrocytes and astrocytes, and (ii) determine the relative contribution of these cells to the neurogenic potential of embryonic, postnatal and adult GZ tissue. In GFAP-TK mice, dividing GFAP-expressing cells are ablated by the antiviral agent ganciclovir (GCV). Treatment with GCV in vitro completely (>99%) eliminated neurospheres formation from postnatal and adult, but not from early embryonic, peri-ventricular GZ tissue of transgenic (but not non-transgenic) mice. Multipotent neurospheres were also derived from primary astrocyte cultures prepared from GZ tissue, and GCV prevented neurosphere formation when using GFAP-TK tissue. Various control experiments were conducted, including use of clonal conditions, to show that GFAP-expressing cells are NSCs rather than support cells. We further show that some but not all GFAP-expressing cells have NSC potential and provide evidence that GFAP-expressing radial glia, but not stellate astrocytes are NSCs. We conclude that a GFAP-expressing cell is the predominant NSC in postnatal and adult, but not early embryonic, GZ. We propose that multipotent GFAP-expressing NSCs in adult GZ derive from GFAP-expressing neurogenic radial glia in postnatal GZ. Supported by: NIH NS07479 and MH65766

Appendix C

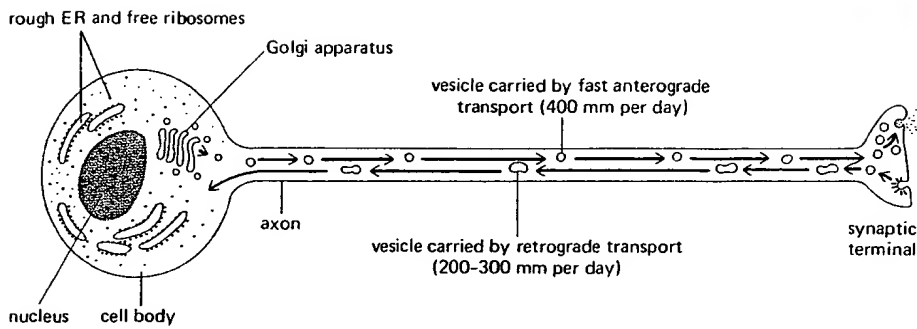


Figure 19-6 A neuron viewed schematically as a secretory cell in which the site of secretion (the axon terminal) lies at a great distance from the site of macromolecular synthesis (the cell body). This mode of organization creates a need for a rapid axonal transport mechanism. The diagram is not meant to imply that all synaptic vesicles have to be transported from the cell body; in most neurons, synaptic vesicles are formed largely by local recycling of membrane in the axon terminal.

Figure 19-20). Molecules present in the extracellular medium surrounding the axon terminal are liable to be captured in these endocytic vesicles and thereby carried back from the axon terminal to the cell body. Thus the biosynthetic machinery in the cell body can sample conditions at the axon terminal and make an appropriate response, as we shall see later (see p. 1119).

Retrograde transport is extremely useful to neuroanatomists, who routinely exploit it to trace neural connections, as explained in Figure 19-7.

Neurons Are Surrounded by Various Types of Glial Cells⁷

All neural tissue, both peripheral and central, consists of two major classes of cells. Neurons play the star role, but they are outnumbered, by about 10 to 1 in the mammalian brain, by a supporting cast of **glial cells**. The glial cells surround neurons (both their cell bodies and their processes) and occupy the spaces between them. The best understood are the *Schwann cells* in vertebrate peripheral nerves and the *oligodendrocytes* in the vertebrate central nervous system, which both wrap themselves around axons to provide electrical insulation in the form of a *myelin sheath* (see p. 1073, below). The three other types of glial cell in the central nervous system are microglia, ependymal cells, and astrocytes (Figure 19-8). The *microglia* belong in a class apart: they are functionally akin to macrophages (see p. 974) and, like them, originate from hemopoietic tissue. With this exception, all the glial cells share a common embryonic origin with the neurons with which they are associated; unlike most neurons, however, they are not as a rule electrically excitable. Moreover, whereas neurons cannot divide after they have differentiated, most glial cells remain capable of dividing throughout life.

Ependymal cells line the internal cavities of the brain and spinal cord (see Figure 19-8), and their epithelial arrangement is a memento of the origin of the central nervous system from an epithelial tube (see p. 1109).

Astrocytes (see Figure 19-8) are the most plentiful and diverse of the glial cells but also the most enigmatic: their functions are still largely uncertain, although it seems clear that they play an important part in guiding the construction of the

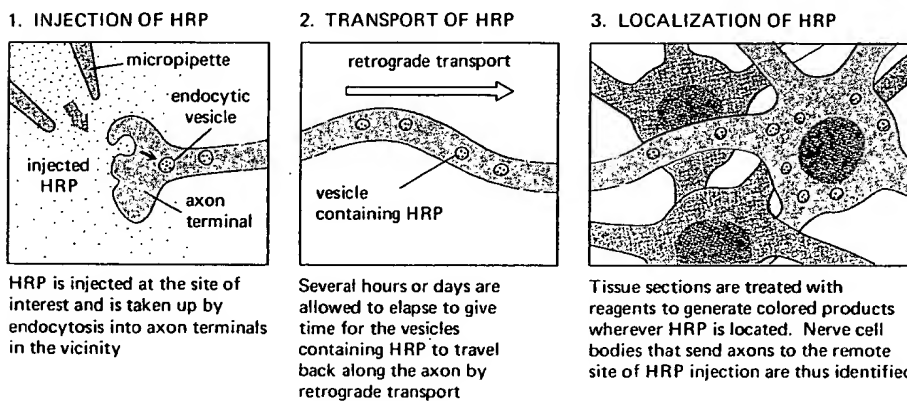


Figure 19-7 How fast retrograde transport is exploited to identify and locate remote nerve cell bodies whose axons project to a given site of interest. The enzyme horseradish peroxidase (HRP) is the most widely used tracer molecule for this purpose, since it can be detected in very small quantities by the colored products of the reaction that it catalyzes.

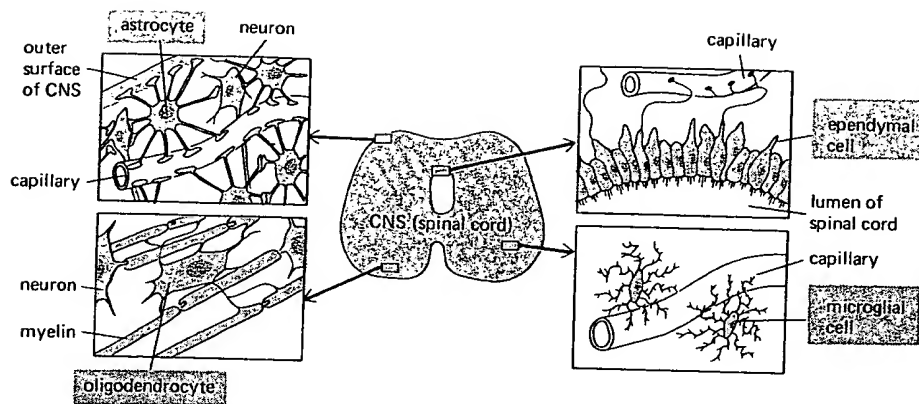


Figure 19-8 The four major classes of glial cells in the vertebrate central nervous system. The glial cells are shown in color. Astrocytes, which are the most plentiful, have many radiating processes. Some of these processes end on the surfaces of neurons; others, with expanded tips, go to form the external surface layer of the CNS and the wrapping that surrounds blood vessels in the CNS, collaborating with endothelial cells of the blood capillaries to create the blood-brain barrier. Ependymal cells form the ciliated epithelial lining of the central cavity of the CNS and, like astrocytes, often have processes ending on blood vessels. Oligodendrocytes form insulating sheaths of myelin around axons in the CNS. Microglial cells are related in function and origin to macrophages and are involved in reactions to tissue damage and infection; they tend to be found in the neighborhood of blood vessels.

nervous system (see p. 1110) and in controlling the chemical and ionic environment of the neurons. Thus one type of astrocyte extends processes that are expanded into "endfeet," which, linked by junctional complexes such as one finds in epithelia (see p. 792), form a sealed barrier at the external surface of the central nervous system. They also extend processes that form similar endfeet on blood vessels, where they induce the endothelial cells (in the case of capillaries and venules) to become sealed together by unusually well-developed tight junctions so as to form the *blood-brain barrier*. This barrier prevents water-soluble molecules from passing into brain tissue from the blood unless they are specifically carried by transport proteins in the plasma membranes of the endothelial cells that line the vessels. The neurons thus occupy a sheltered and controlled environment, on which the molecular machinery for electrical signaling is critically dependent.

Summary

Nerve cells, or neurons, are exceptionally elongated cells that convey electrical signals in the form of action potentials—traveling waves of electrical excitation. Typically, several branching dendrites and a single long axon project from the nerve cell body. Signals are usually received on the dendrites and cell body, sent out along the axon, and passed on to other cells at chemical synapses. Here the electrical signal in the presynaptic axon terminal triggers the secretion of neurotransmitter, which provokes an electrical change in the postsynaptic cell.

The neuron can be viewed as a secretory cell that releases its secretion—the neurotransmitter—at a very large distance from the cell body, where macromolecules are synthesized. Newly synthesized membrane and proteins for secretion are exported along the axon and dendrites by fast axonal transport, in which small membrane vesicles are propelled along tracks formed by microtubules. The microtubules and other non-membrane-bound components of the neuronal cytosol are exported from the cell body by a quite different, slow axonal transport mechanism. Fast axonal transport also operates in a retrograde direction, conveying membrane vesicles back from the axon terminals to the cell body.

Neurons are surrounded by glial cells, which help in various ways to control the chemical and electrical environment of the neurons.

Voltage-gated Ion Channels and the Action Potential^{3,4,8}

As discussed in Chapter 6, the voltage difference across a cell's plasma membrane—the **membrane potential**—depends on the distribution of electric charge (see p. 314). Charge is carried back and forth across the nerve cell membrane by small organic ions—chiefly Na^+ , K^+ , Cl^- , and Ca^{2+} —which traverse the lipid bilayer by passing through selective ion channels formed by specific transmem-